

Patent claims

1. An isolated polynucleotide from coryneform bacteria,
comprising a polynucleotide sequence which codes for
the menE gene, chosen from the group consisting of
- a) polynucleotide which is identical to the extent of
at least 70% to a polynucleotide which codes for a
polypeptide which comprises the amino acid sequence
of SEQ ID No. 2,
- b) polynucleotide which codes for a polypeptide which
comprises an amino acid sequence which is identical
to the extent of at least 70% to the amino acid
sequence of SEQ ID No. 2,
- c) polynucleotide which is complementary to the
polynucleotides of a) or b), and
- d) polynucleotide comprising at least 15 successive
nucleotides of the polynucleotide sequence of a),
b) or c),
- the polypeptide preferably having the activity of O-
succinylbenzoic acid CoA ligase.
2. A polynucleotide as claimed in claim 1, wherein the
polynucleotide is a preferably recombinant DNA which is
capable of replication in coryneform bacteria.
3. A polynucleotide as claimed in claim 1, wherein the
polynucleotide is an RNA.
4. A polynucleotide as claimed in claim 2, comprising the
nucleic acid sequence as shown in SEQ ID No. 1.
5. A DNA as claimed in claim 2 which is capable of
replication, comprising
- (i) the nucleotide sequence shown in SEQ ID No. 1, or

- (ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or
- (iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally
- (iv) sense mutations of neutral function in (i).
6. A DNA as claimed in claim 5 which is capable of replication,
wherein
the hybridization is carried out under a stringency corresponding to at most 2x SSC.
7. A polynucleotide sequence as claimed in claim 1, which codes for a polypeptide which comprises the amino acid sequences shown in SEQ ID No. 2.
8. A coryneform bacterium in which the menE gene is attenuated, in particular eliminated.
9. The integration vector pCR2.1menEint, which
- 9.1. carries an internal fragment of the menE gene 520 bp in size,
- 9.2. the restriction map of which is reproduced in figure 1, and
- 9.3. which is deposited in the E. coli strain Top10/pCR2.1menEint under no. DSM 14080 at the Deutsche Sammlung für Mikroorganismen und Zellenkulturen].
10. A process for the fermentative preparation of L-amino acids, in particular L-lysine,
which comprises
carrying out the following steps:

- a) fermentation of the coryneform bacteria which produce the desired L-amino acid and in which at least the menE gene or nucleotide sequences which code for it are attenuated, in particular eliminated;
- 5 b) concentration of the L-amino acid in the medium or in the cells of the bacteria, and
- c) isolation of the L-amino acid.
- 10 11. A process as claimed in claim 10, wherein bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally enhanced are employed.
- 15 12. A process as claimed in claim 10, wherein bacteria in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly eliminated are employed.
- 20 13. A process as claimed in claim 10, wherein the expression of the polynucleotide(s) which code(s) for the menE gene is attenuated, in particular eliminated.
- 25 14. A process as claimed in claim 10, wherein the catalytic properties of the polypeptide (enzyme protein) for which the polynucleotide menE codes are reduced.
15. A process as claimed in claim 10, wherein for the preparation of L-amino acids, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of
- 15.1 the dapA gene which codes for dihydrodipicolinate synthase,
- 15.2 the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase,

- 15.3 the tpi gene which codes for triose phosphate isomerase,
- 15.4 the pgk gene which codes for 3-phosphoglycerate kinase,
- 5 15.5 the zwf gene which codes for glucose 6-phosphate dehydrogenase,
- 15.6 the pyc gene which codes for pyruvate carboxylase,
- 10 15.7 the mqo gene which codes for malate-quinone oxidoreductase,
- 15.8 the lysC gene which codes for a feed-back resistant aspartate kinase,
- 15.9 the lysE gene which codes for lysine export,
- 15 15.10 the hom gene which codes for homoserine dehydrogenase
- 15.11 the ilvA gene which codes for threonine dehydratase or the ilvA(Fbr) allele which codes for a feed back resistant threonine dehydratase,
- 20 15.12 the ilvBN gene which codes for acetohydroxy-acid synthase,
- 15.13 the ilvD gene which codes for dihydroxy-acid dehydratase, and
- 15.14 the zwal gene which codes for the Zwal protein
- 25 is or are enhanced or over-expressed are fermented.
16. A process as claimed in claim 10, wherein for the preparation of L-amino acids, coryneform microorganisms

in which at the same time one or more of the genes
chosen from the group consisting of

16.1 the pck gene which codes for phosphoenol
pyruvate carboxykinase,

5 16.2 the pgi gene which codes for glucose 6-
phosphate isomerase,

16.3 the poxB gene which codes for pyruvate oxidase,
and

16.4 the zwa2 gene which codes for the Zwa2 protein
10 is or are attenuated are fermented.

17. A coryneform bacterium which contains a vector which
carries parts of the polynucleotide as claimed in
claim 1, but at least 15 successive nucleotides of the
sequence claimed.

15 18. A process as claimed in one or more of the preceding
claims, wherein microorganisms of the species
Corynebacterium glutamicum are employed.

19. A process for discovering RNA, cDNA and DNA in order to
isolate nucleic acids, or polynucleotides or genes which
20 code for O-succinylbenzoic acid CoA ligase or have a high
similarity with the sequence of the menE gene, which
comprises employing the polynucleotide comprising the
polynucleotide sequences as claimed in claims 1, 2, 3 or 4
as hybridization probes.

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